Br. J. clin. Pharmac. (1978), **5**, 431–436

INCREASED PROSTAGLANDINS E_2 AND $F_{2\alpha}$ IN HUMAN SKIN AT 6 AND 24 H AFTER ULTRAVIOLET B IRRADIATION (290–320 nm)

A. KOBZA BLACK, M.W. GREAVES, C.N. HENSBY & N.A. PLUMMER
Professorial Unit, Institute of Dermatology, Homerton Grove, London E9 6BX

1 Abdominal skin of 25 human subjects was irradiated with three times its minimal erythema dose of ultraviolet (u.v.) B radiation. Erythema appeared after 2 h, was of moderate degree at 6 h, and maximal at 24 and 48 h.

2 Exudate was recovered by a suction bulla technique from the subject's normal and erythematous skin either at 6, 24 or 48 h after irradiation.

3 Superfusion cascade bioassay of exudate showed increased prostaglandin (PG)-like activity, measured in PGE_2 equivalents, at 6 and 24 h after irradiation. The maximum rise was at 24 h, coinciding with the peak of the erythema. However, prostaglandin concentrations were not significantly above that of controls at 48 h when the erythema was still maximal.

4 Radioimmunoassay for $PGF_{2\alpha}$ yielded values in close agreement with the bioassay results.

5 Gel partition chromatography, after conversion of extracted residues from the exudate to ^{14}C methyl esters, showed increased incorporation of radioactivity at 6 h, maximal at 24 h. At 48 h there was no significant difference from control levels. The major increased activity, maximal at 24 h, co-chromatographed with arachidonic acid and PGE_2 and $PGF_{2\alpha}$. The materials provisionally identified as the latter three compounds also co-chromatographed with their corresponding methyl esters on t.l.c., although the arachidonic acid-like material contained at least two peaks. No evidence was obtained for the 1 or 3 series of PGs. On g.l.c. the material obtained from the $PGF_{2\alpha}$ zone co-chromatographed with authentic $PGF_{2\alpha}$ as the methyl ester trimethylsilyl ether. The PGE_2 zone converted to methyl ester *n*-butyloxime trimethylsilyl ether also co-chromatographed with authentic PGE_2, but with some distinct shouldering.

6 The evidence presented provides support for the presence of increased amounts of arachidonic acid, PGE_2 and $PGF_{2\alpha}$-like compounds during the first 24 h of u.v.B erythema, with concordance of the erythema and prostaglandin levels. At 48 h this relationship could not be demonstrated.

Introduction

The involvement of prostaglandins in the reaction to ultraviolet irradiation of human skin is suggested by the increase in PGE_1 like activity (Sondergaard & Greaves, 1970) and by the ability of prostaglandin synthetase inhibitors to delay and diminish the inflammatory response (Gruber, Ridolfo, Nickander & Mikulaschek, 1971; Snyder & Eaglstein, 1974a, b). We have studied the nature of this prostaglandin-like activity and its relationship to the time course of the inflammation.

Methods

Subjects

Seven female and eighteen male adult human volunteers of an age range 16–67 years (mean ± s.e. mean 41.5 ± 3.3) were irradiated on untanned clinically normal lower abdominal skin. The subjects, some with localized skin disease, had no history of photosensitivity and were not taking oral antihistamines, corticosteroids or non-steroidal anti-inflammatory agents. The Institute's Ethical Committee approved, and each subject gave prior informed consent.

Irradiation

Four F.S. 20 Westinghouse sun tubes 30 cm from the subject (major emission 290–320 nm) with an intensity of 430 $\mu W\ cm^{-2}$ below 320 nm at 30 cm were used, for irradiation. Each subject's abdomen was irradiated with three times the minimal erythema dose (M.E.D.), on an area of approximately 120 cm^2.

The erythema was assessed visually: 0, no

erythema, 1, minimal, 2, moderate, 3, deep red. Oedema was never observed.

Collection of exudate

Exudate was obtained by placing perspex cups, each containing a dome shaped diaphragm with five 6 mm diameter holes, onto the skin (Black, Greaves, Hensby & Plummer, 1976). Continuous suction at 200 mm Hg below atmospheric pressure produced suction bullae due to separation of the dermis and epidermis (Kiistala, 1968). The exudate from each bulla (approximately 50 µl) was aspirated using a needle and a polystyrene syringe, and stored at −20°C until analysed. Control samples were obtained simultaneously from contralateral unirradiated skin.

Analysis

Bioassay. Some of the exudates diluted 1:5 in Krebs solution were examined for smooth muscle contracting activity by the superfusion cascade bioassay method of Vane (1964). Prostaglandin-like compounds caused contraction of the rat stomach fundic strip and rat colon in the presence of indomethacin and selective antagonists (Palmer, Piper & Vane, 1973). Atropine sulphate was substituted for hyoscine in the superfusing fluid (347 ng ml^{-1}; 5 ml min^{-1}). The assay system could detect 2.5 ng PGE_2 ml^{-1}.

Radioimmunoassay. The exudates were also examined for $PGF_{2\alpha}$-like activity by a double antibody radioimmunoassay. The antiserum showed minimal cross-reactivity to the 1 and 2 series of prostaglandins A, B, E and F_β as well as 15-keto-PGE_2 and 15-keto-$PGF_{2\alpha}$. However the prostaglandins D_1 and D_2 showed approximately 3% cross-reactivity, while $PGF_{1\alpha}$ showed total cross-reactivity. The assay could detect 3 pg of authentic $PGF_{2\alpha}$.

Extraction and conversion to ^{14}C methyl esters. Exudates from each group were pooled, diluted three times with distilled water and the pH adjusted to approximately 4 with hydrochloric acid (0.1 mol l^{-1}). After organic solvent extraction (three times with redistilled ethyl acetate) the pooled organic phases were taken to dryness under reduced pressure at 40–45°C and the residue vaccum-desiccated. The resulting residues were redissolved in 0.5 ml methanol and treated with 5 ml of ethereal [^{14}C]-diazomethane for 5 min at room temperature. These solutions were taken to dryness and vacuum-desiccated. The procedure was repeated with non-radioactive diazomethane. After vacuum desiccation the resulting residues were stored at −20°C until subjected to straight phase gel partition column chromatography.

Straight phase gel partition column chromatography. A glass column (450 x 12 mm) was silanized by treatment for 2 h at room temperature with 5% (v/v) dichloro-dimethylsilane (Sigma) in toluene. Excess silanizing reagent was removed by successive washing with chloroform and methanol. After drying, the column was plugged at the

Table 1 The chromatographic properties of arachidonic, oleic and prostaglandin methyl esters on silica gel G thin-layer chromatography in the absence and presence of 3% $AgNO_3$, and on Lipidex 5000 straight phase gel partition column chromatography.

Compound	*Mean R_F (methyl ester) F VI solvent*		*Mean % bed volume Lipidex 5000 (heptane:chloroform 80:20 v/v)*
	No $AgNO_3$	*3% $AgNO_3$*	
PGE_1	0.27	0.27	5.5†
PGE_2	0.28	0.17	5.8†
$PGF_{1\alpha}$	0.13	0.12	10.4†
$PGF_{2\alpha}$	0.12	0.05	8.7†
Arachidonic acid	0.85	0.32	1.1†
Oleic acid	0.88	0.77	1.0†
15-keto-$PGF_{2\alpha}$	0.39	0.19	4.0†
15-keto-13, 14-dihydro-$PGF_{2\alpha}$	0.42	0.22	2.0*
15-keto-13, 14-dihydro-PGE_2	0.73	0.47	1.6*
15-keto-PGE_2	0.72	0.56	2.6*
PGD_1	0.61	0.37	Not tested
PGD_2	0.59	0.20	3.7*
PGA_2	0.75	0.50	1.6*

* Assayed by g.l.c.
† Assayed by liquid scintillation counting.

base with silanized glass wool and packed with Lipidex 5000 gel (Packard) as described previously (Brash & Jones, 1974; Hensby, 1975). Prior to packing, the gel had been equilibrated with heptane-chloroform (80:20 v/v), and after equilibration *in situ* before use, excess gel was removed to leave 50 ml. A constant pressure head at the top of the column maintained a flow rate of 8–10 ml h^{-1}. Samples to be chromatographed (as the methyl esters) were vacuum-desiccated for at least 15 min immediately prior to dissolution in 100 µl chloroform. After adding heptane (400 µl), the sample was vortexed and loaded on to the column. A further wash (500 µl of column eluent mixture) was loaded on to the column.

The column effluent was led via narrow bore Teflon tubing (L.K.B.) to the drop-counting head of an L.K.B. Ultrorac fraction collector. Fractions of 5 ml (10% bed volume) were collected until the 2000% bed volume point was reached. The elution profile of biological extracts was obtained by liquid scintillation counting of a 200 µl aliquot from alternate odd-numbered fractions. The elution profile of authentic prostaglandins (as their methyl esters) was obtained either by liquid scintillation counting (where radio-labelled ^{3}H or ^{14}C compounds were available) or by g.l.c. with flame ionization detection (Table 1).

From the elution profile of the biological extracts it was possible to bulk several fractions into corresponding zones. These were taken to dryness and re-dissolved in 1 ml of methanol. Liquid scintillation counting of two 10 µl aliquots determined the recovery of radioactivity.

Thin-layer chromatography. Samples and authentic prostaglandin methyl esters were spotted separately on glass plates (Anderman) of neutral silica gel (100 x 200 x 0.25 mm). The plates were developed to 15 cm in closed equilibrated glass tanks containing the F VI solvent (Anderson, 1969). This comprised ethyl acetate, acetone and glacial acetic acid (180:20:1 by volume). The R_F values of authentic prostaglandins on the above t.l.c. systems is shown in Table 1.

Liquid scintillation counting. This was performed on an L.K.B. 1210 liquid scintillation counter using 8 ml of scintillant (toluene–2 ethoxyethanol 1500:900 v/v, P.P. O., 10.5, naphthalene, 112.5 w/w) per vial.

Preparation of derivatives for gas-liquid chromatography. Methyl esters were prepared as described above. Trimethylsilyl ethers were prepared by treating the desiccated residue (either authentic or biological samples) with N, N-bis (trimethylsilyl)-trifluoracetamide (BSTFA: Sigma) using the method of Thompson, Los & Horton (1970). The *n*-butyloxime derivatives were prepared by reacting the desiccated residue with 100 µl *n*-butyloxime hydrochloride in anhydrous pyridine at 60°C for 90 min.

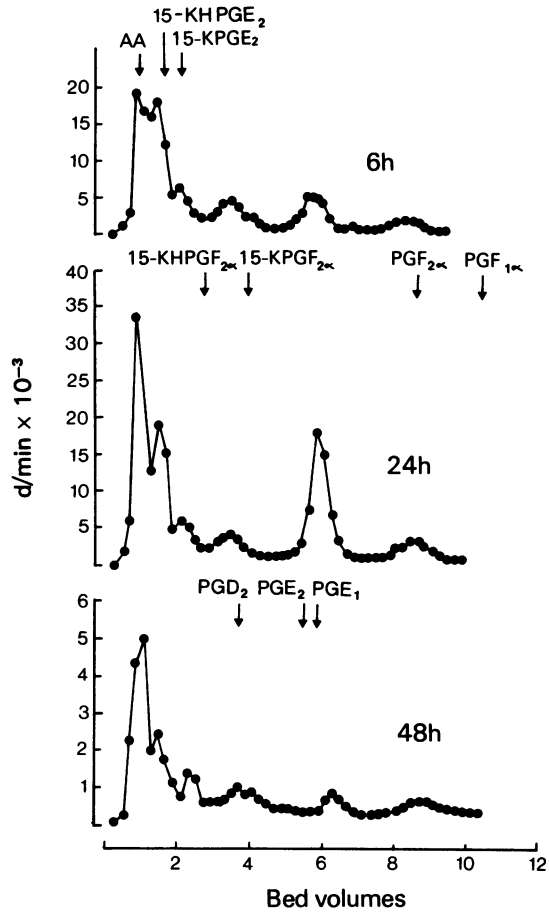

Figure 1 Elution profiles obtained from a Lipidex-5000 gel partition column of samples obtained at 6, 24 and 48 h after u.v.B irradiation. The samples were converted to ^{14}C methyl esters prior to chromatography and the column (50 ml bed volume: 5.0 ml fractions) eluted with heptane-chloroform (80:20 v/v).

Gas-liquid chromatography. A Pye series .104 gas chromatograph equipped with a 3 m x 4 mm glass column was used. The column stationary phase was 3% OV-1 on Chromsorb-W (Pye Unicam). The oven temperature was 260–265°C and the carrier gas (nitrogen) flow rate was 35–40 ml min^{-1}. Samples were injected in 5 µl of either n-hexane (fatty acid methyl esters) or BSTFA (authentic prostaglandins and biological extracts). The gas chromatograph was calibrated for carbon values of authentic saturated fatty acid methyl esters (C16 to C24) using the method of Bergstrom, Rhyage, Samuelsson & Sjovall (1963) and authentic prostaglandins E_1, E_2 and $F_{2\alpha}$ derivatives.

Solvents and chemicals. All solvents were of analytical grade or redistilled before use. The chloroform was redistilled over anhydrous calcium chloride and 1% (v/v) of absolute ethanol was added to the redistillate to stabilize it.

Fatty acids were obtained from Sigma. Pyridine and diazald were obtained from Aldrich. [^{14}C]-Diazald, [^{14}C]-arachidonic acid, [^{14}C]-oleic acid, [^{3}H]-sodium borohydride and high specific activity [^{3}H]-prostaglandins E_1, E_2 and $F_{2\alpha}$ were obtained from the Radiochemical Centre, Amersham. Low specific activity [^{3}H]-prostaglandins $PGF_{1\alpha}$, $PGF_{2\alpha}$ and $PGF_{2\beta}$ were obtained by reduction of the corresponding PGE compound with [^{3}H]-sodium borohydride. Donkey anti-rabbit precipitating serum was obtained from Wellcome Laboratories.

Results

Erythema

The erythema resulting from exposure to 3 M.E.D. u.v.B. first appeared 2 h after irradiation, was of moderate degree (scale 2) at 6 h and was maximum (Scale 3) at 24 and 48 h.

Prostaglandins

Prostaglandins and related compounds were assayed by the cascade bioassay as PGE_2 equivalents. Table 2 shows that a statistically significant rise occurred at 6 and 24 h after irradiation. The maximum rise was at 24 h and this coincided with the peak of the erythema. However, at 48 h when the erythema was still maximal the concentration of prostaglandin-like activity was not significantly elevated above control levels.

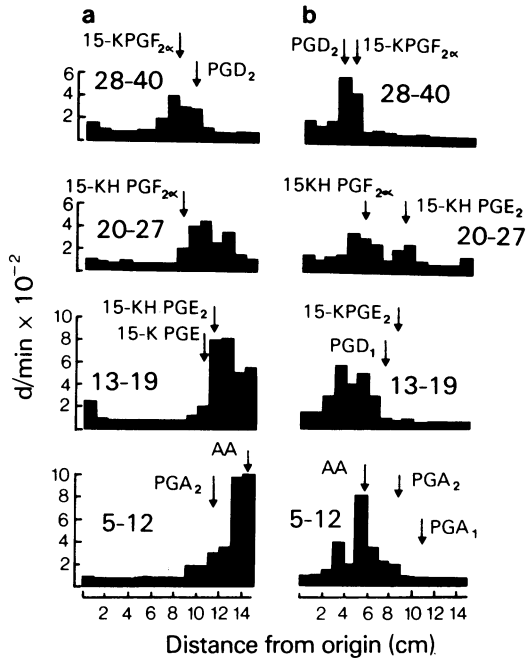

Figure 2 Thin layer chromatography on silica gel G in (a) the absence and (b) the presence of 3% silver nitrate of various fractions (5–12, 13–19, 20–27, 28–40) obtained from the Lipidex 5000 column profile of the 24 h u.v.B sample (Figure 1). The chromatography solvent was ethyl acetate: acetone: glacial acetic acid (180:20:1 by volume).

Radioimmunoassay of the exudate samples for $PGF_{2\alpha}$ yielded values agreeing closely with the bioassay results (Table 2).

The pooled exudate samples from control, 6, 24 and

Table 2 The mean ± s.e. mean levels of bioassayable PGE_2 equivalents and radioimmunoassayable $PGF_{2\alpha}$ equivalents in exudate from normal human skin and skin 6, 24 and 48 h after 3 M.E.D. of u.v.B irradiation

	Normal	*Time after u.v.B irradiation (h)*		
		6	*24*	*48*
Bioassay				
Prostaglandins (PGE_2 equivalents ng ml^{-1})	31.3 ± 12.6	100 ± 18	244 ± 90	56 ± 19
	$n=10$	$n=4$	$n=4$	$n=4$
		$P<0.02$	$P<0.005$	$P>0.30$
Radioimmunoassay				
$PGF_{2\alpha}$ equivalents ng ml^{-1})	21.3 ± 4.8	43.8 ± 16.5	56.7 ± 9.0	22.7 ± 4.7
	$n=27$	$n=9$	$n=11$	$n=8$
		$P>0.10$	$P<0.0005$	$P>0.40$

n = number of samples
P values show significance relative to normal.

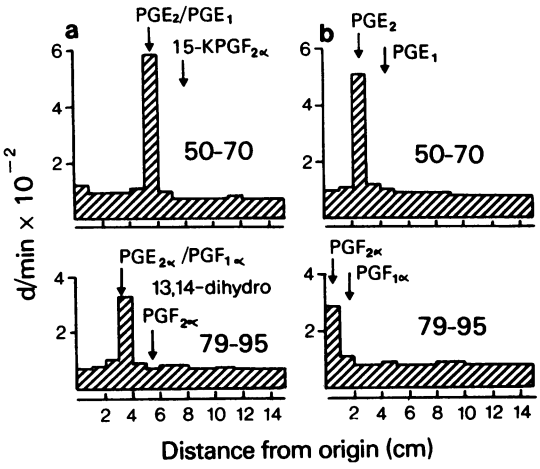

Figure 3 Thin layer chromatography on silica gel G of fractions 50–70 and 79–95 obtained from the Lipidex 5000 column profile of the 24 h u.v.B sample. The profiles were obtained (a) in the absence of and (b) in the presence of 3% silver nitrate. Both types of t.l.c. plate were chromatographed in ethyl acetate: acetone: glacial acetic acid (180:20:1 by volume).

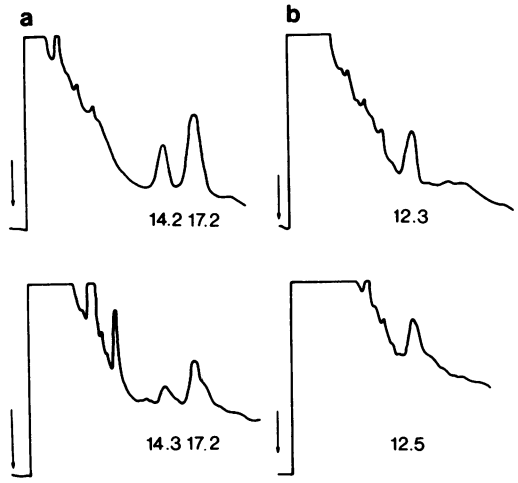

Figure 4 Gas liquid chromatography–flame ionization traces obtained for: (a) Authentic PGE_2 methyl ester, *n*-butyloxime, trimethylsilyl ether (upper trace) and the corresponding derivative of fractions 50–70 obtained from the Lipidex 5000 column profile of the 24 h u.v.B sample (lower trace). (b) Authentic $PGF_{2\alpha}$ methyl ester, trimethylsilyl ether (upper trace) and the corresponding derivative of fractions 79–95 obtained from the Lipidex 5000 column profile of the 24 h u.v.B sample (lower trace). The sample was injected at the arrow. The numbers indicate retention time in min.

48 h were extracted and the residues converted to ^{14}C methyl esters prior to gel partition chromatography (Figure 1). Compared with control levels the incorporation of radioactivity was elevated at 6 h and maximal at 24 h, but the 48 h levels were not raised compared with control vlaues.

Several peaks of radioactivity were eluted from the Lipidex 5000 columns. However, the major increases, which were maximum at 24 h, were in material co-chromatographing with arachidonic acid, and PGE_2 and $PGF_{2\alpha}$. Further characterization was obtained by t.l.c. in the presence and absence of 3% silver nitrate using the F VI solvent of Anderson (1969).

The arachidonic acid-like peak (Figure 2) contained at least two compounds, one of which co-chromatographed with authentic arachidonic acid methyl ester. The material provisionally identified as PGE_2 and $PGF_{2\alpha}$ (from Lipidex 5000) also co-chromatographed with the corresponding authentic methyl esters on t.l.c. (Figure 3). No evidence was obtained for either the 1 or 3 series of each prostaglandin.

On g.l.c. the material obtained from the $PGF_{2\alpha}$ zone co-chromatographed with authentic $PGF_{2\alpha}$, as the methyl ester trimethylsilyl ether (Figure 4). However, the PGE_2 zone when subjected to g.l.c. as the methyl ester *n*-butyloxime trimethylsilyl ether was found to co-chromatograph with authentic PGE_2 but also had distinct unidentified shoulders.

Discussion

Skin erythema due to u.v.B. irradiation has been said to result from increased local protaglandin formation. Sondergaard & Greaves (1970) found fatty acid material in perfusates from human skin following u.v. irradiation, from an unfiltered mercury-quartz source. Eaglstein & Marsico (1975) considered that the ability of indomethacin to inhibit erythema in response to u.v.B irradiation of human skin suggested the involvement of increased prostaglandin activity in the observed reactions. Irradiation of guinea-pig skin with u.v.B raised PGE levels and topical application of indomethacin immediately after irradiation suppressed this increase and the erythema (Snyder, 1976).

Using a suction bullae technique to obtain inflammatory exudate from inflamed human skin at different times after irradiation with 3 M.E.D. of u.v.B we have confirmed the presence of increased prostaglandin E_2-like activity and also demonstrated the presence of $PGF_{2\alpha}$ and arachidonic acid-like fatty acid material in the reddened skin. Because of the presence of increased concentrations of prostaglandin activity in the irradiated skin and the earlier findings that indomethacin, an inhibitor of PG biosynthesis, suppresses u.v.B erythema, it is tempting to speculate

that sunburn erythema is mediated at least partly by prostaglandins. The findings in skin at 48 h after irradiation are inconsistent with the view that the intense erythema at this time is solely attributable to a direct action of PG mediators since the activity measured at this time was only slightly higher than control values. It is of some interest that Snyder (1976) obtained similar results in the guinea pig.

The apparent dissociation of erythema and prostaglandin activity can be explained in various ways. The intensity of inflammation was measured by visual assessment of the degree of erythema. Serial measurements of blood flow in the irradiated skin might have provided a better correlation with prostaglandin levels. A sustained increase in levels of prostaglandin is unnecessary since a single bolus of prostaglandin E produces erythema persisting for several hours (Greaves & Sondergaard, 1971). Blood vessels contain prostaglandin synthetase (Tuvemo & Wide, 1973) and prostagandins from this source, which might not be detected by the suction bulla technique, could regulate blood vessel tone independantly of prostaglandins formed from other cellular elements in the skin in response to u.v.B irradiation. It is also possible that increased prostaglandin activity in irradiated skin is irrelevant to the pathogenesis of the erythema, and might even subserve a protective role; perhaps it reduces the vulnerability of the epidermal cells to the harmful actions of u.v.B on the genome, since the E prostaglandins can inhibit cell replication (Thomas, Philpott & Jaffe, 1974). u.v.B erythema might involve other products of arachidonic acid metabolism including thromboxanes, PGD_2 or PGI_2, which we have not measured.

Our results suggest that the realtionship between increased PG activity and u.v.B erythema may be more complex than has previously been thought. A more detailed analysis, based on GC-MS data for products of the arachidonic acid cascade at a wide range of times after irradiation in the presence and absence of indomethacin, coupled with simultaneous measurements of other putative mediators and of blood flow in the irradiated skin should do much to elucidate these relationships. Nevertheless the fact that PGs are increased up to 24 h suggests that they contribute to at least the early part of the response.

This work was supported by grants from the Medical Research Council, The Wellcome Trust and the Sir Herbert Dunhill Trust. We thank Miss M. Davison for skilled technical assistance. Grateful thanks are due to Professor E.W. Horton (Edinburgh) and Dr J. Pike (Upjohn Co.) for their generous gifts of antiserum and prostaglandins respectively.

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(Received May 12, 1977)